Operational Screening and Breeding Program to Develop White Pine Blister Rust Resistance for Five-Needle Pines in Oregon and Washington: Current Status

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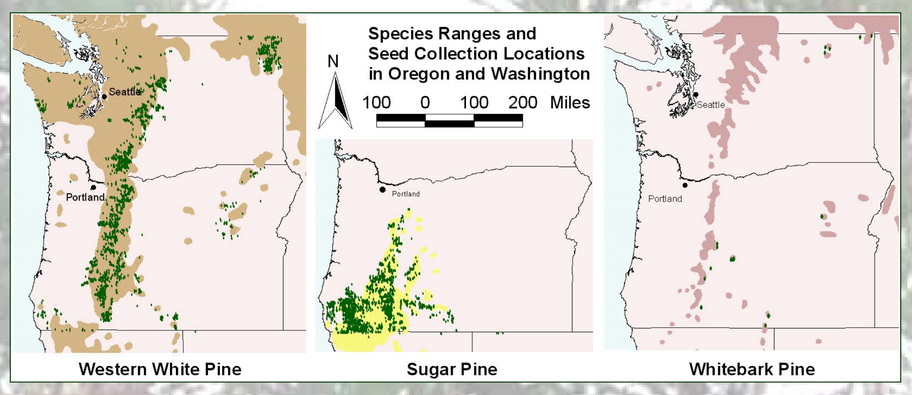
Whitebark pine at Crater Lake National Park

Introduction: Throughout much of western North America, native populations of five-needle pines have been dramatically reduced by the disease white pine blister rust (WPBR), caused by the introduced pathogen *Cronartium ribicola*. Land managers are reluctant to plant non-resistant western white pine (WWP) and sugar pine (SP), thus continuing the decline in these species. Genetic resistance appears to offer the best avenue for restoring and sustaining five-needle pines in North American ecosystems.

In Region 6 (Oregon and Washington), the USDA Forest Service (FS) began an operational program to develop blister rust resistant populations of five-needle pines in the late 1950's. This program is based at Dorena Tree Improvement Center (DTIC). Artificial inoculation and evaluation of WWP and SP seedlings (and more recently, whitebark pine seedlings) is the core of the resistance testing. Several types of resistance have been identified, including major gene resistance (Kinloch *et al*. 1999) and some forms of partial resistance. Progeny of more than 4900 WWP and 4500 SP phenotypic selections have been evaluated since the program's inception.

Progeny testing at Happy Camp, CA



Large sugar pine on Bureau of Land Management lands

Blister rust mortality in an operational screening trial at Dorena Tree Improvement Center.

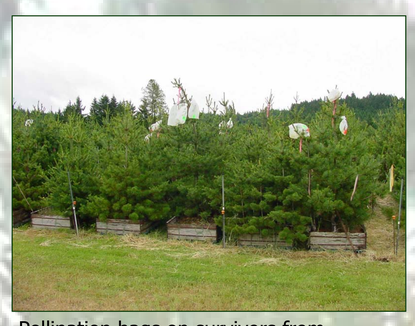
Objective: Restore and sustain populations of five-needle pines that have durable blister rust resistance

Methods:

- Operational Screening: Two-year-old progeny of WWP and SP phenotypic selections are artificially inoculated with *C. ribicola* and evaluated for development of needle lesions, stem symptoms (cankers and bark reactions), and mortality over a period of five years (Sniezko 1996).
- Screening for Major Gene Resistance: Seedlings at the cotyledon or primary needle stage are artificially inoculated with blister rust and are examined for presence of a hypersensitive reaction (HR) in the foliage (Kinloch and Dupper 2002).
- Field Validation Trials: Selected families have been deployed in several series of replicated trials to evaluate long-term effectiveness of resistance 'mechanisms' in natural environments.

Why Plant Pines?

Despite potential losses due to WPBR, five-needle pines can be quite desirable for reforestation. In addition to filling an ecological niche, these native conifers are less susceptible to some insects and root diseases such as laminated root rot and Annosus root rot than Douglas-fir and grand fir (Hadfield *et al.* 1986, Harrington and Wingfield 1998, Fins *et al*. 2001). They are more tolerant of drought and frost than many other conifers (Oliver 1996, Neuenschwander *et al*. 1999). Additionally, planting on low rust hazard sites and using silvicultural tools, such as pruning lower limbs (Hagle *et al.* 1989, Hayes and Stein 1957) have been shown to be effective in helping reestablish five-needle pines.

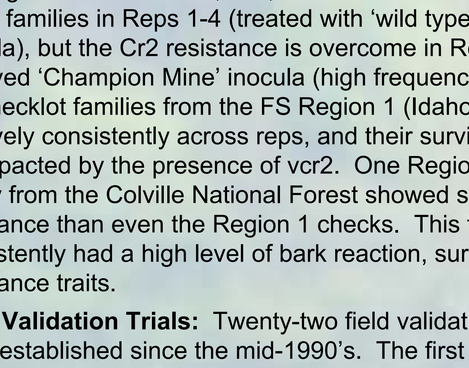


Pollination bags on survivors from operational screening trials at DTIC

Overview of 2002 pollination in one of DTIC's on-site orchard blocks

Results:

- **Screening for Major Gene Resistance:** The most dramatic resistance mechanism in both SP and WWP is a hypersensitive reaction (HR) in the needles controlled by separate, single dominant genes, Cr1 in SP, Cr2 in WWP. Individuals with the resistance allele are usually canker-free, but there are a few geographic areas where there are different strains of rust virulent to Cr1 and Cr2 ('vcr1' and 'vcr2', respectively) (Sniezko *et al*. 2001). HR individuals develop normal stem symptoms after infection with the virulent strain of rust. Screening for major gene resistance has been used to:
  - Examine the geographic distribution of Cr2 in WWP (Sniezko et al. 2001)
  - Retest some open-pollinated families from our operational screening that had a moderate to high (35-65) percentage stem symptom free
    - In WWP it was noted that some of the families with a high percentage of stem symptom free trees do not exhibit HR; several of these half-sib families are being re-evaluated and in the interim are designated 'Mechanism X.'
- **Operational Screening**: Only a low level of resistance has been observed in most open-pollinated progeny of phenotypic selections. Figure 1 shows results from one of the many artificial screening trials; the average survival of the progeny of 110 WWP phenotypic selections was below 10%. Figure 1 also shows genotype x inocula source interaction in the Dorena checklots. These families have a major gene for resistance, Cr2, which contributes to the high survival of the families in Reps 1-4 (treated with 'wild type' or mostly AVCr2 inocula), but the Cr2 resistance is overcome in Reps 5-6, which received 'Champion Mine' inocula (high frequency of vcr2). The full-sib checklot families from the FS Region 1 (Idaho) program performed relatively consistently across reps, and their survival appears not to be impacted by the presence of vcr2. One Region 6 open-pollinated family from the Colville National Forest showed slightly higher resistance than even the Region 1 checks. This family has consistently had a high level of bark reaction, survival, and other resistance traits.
- **Field Validation Trials:** Twenty-two field validation plantings have been established since the mid-1990's. The first of these with moderate rust infection is a 1996 planting of SP and WWP at Happy Camp, CA.
  - SP had a higher percentage of trees with stem symptoms (SS) than WWP at Happy Camp (85.4 vs. 43.2). Highly significant differences for SS% existed between species and among WWP families but not among SP families. Except for the susceptible WWP (control) family, there was no overlap in SS% among the 12 SP and 13 WWP families (Figure 2).
  - There were positive and significant correlations between SS% in DTIC screening and SS% at Happy Camp for SP and WWP (Figure 3).
- Grass Creek is among the earliest plantings (1968-1972) of WPBR-resistant WWP progeny produced by the Region 6 program. Many of the individuals outplanted were the resistant segregants for a major gene for resistance (Cr2). After nearly 30-years' exposure to vcr2, most of the families had cankers, and the number of cankers per tree varied widely by family (Figure 4). Survivor phenotypes include reduced infection frequency, bark reaction, and tolerance.



Survivors from the 1996 western white pine operational screening trial. The two trees in the foreground are control-cross progeny from the FS Region 1 blister rust resistance program, and the two in the background are from a Dorena orchard collection.

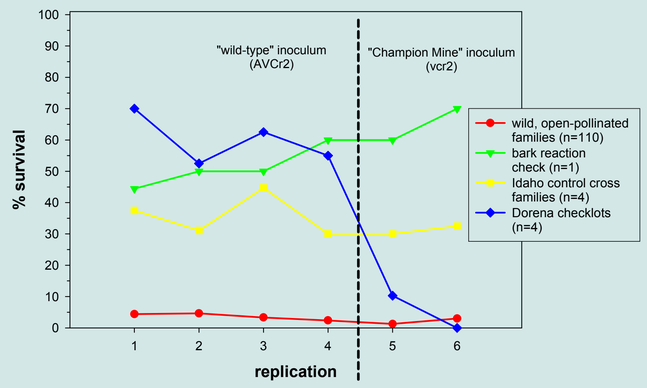


Figure 1. Percent survival for several sources of western white pine included in a 1994 operational screening trial at Dorena

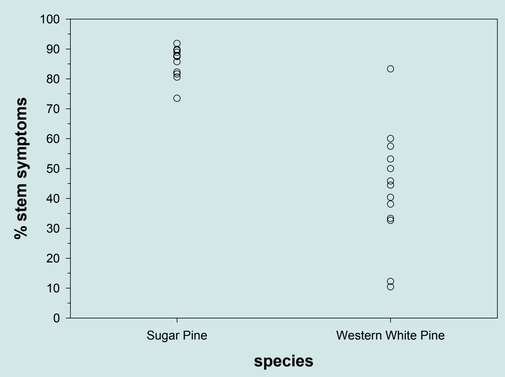


Figure 2. Range of family means for percent stem symptoms at Happy Camp for 12 sugar pine and 13 western white pine families.

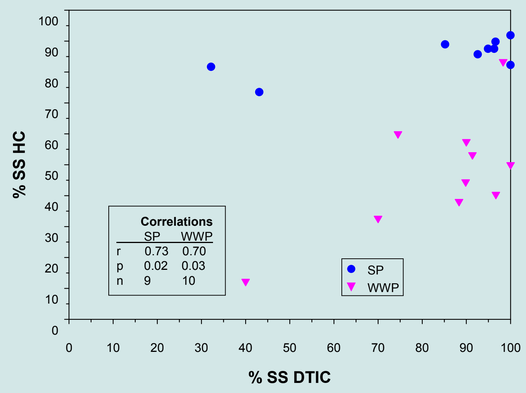


Figure 3. Percent stem symptoms at Happy Camp, CA field planting (%SS HC) vs. percent stem symptoms at DTIC (%SS DTIC) for 9 sugar pine (SP) and 10 western white pine (WWP) families.

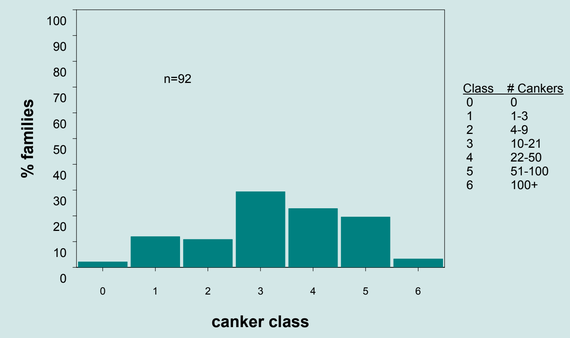


Figure 4. Distribution of family mean number of cankers (canker class) of trees alive in 1997 at Grass Creek

Summary

- **Genetic Variation in WPBR Resistance**
  - Genetic resistance to WPBR in natural populations of Oregon and Washington WWP and SP is in low frequency. However, families outstanding for one or more resistance traits exist.
  - SP is more susceptible to WPBR than WWP. This coupled with long generation intervals will provide a greater challenge to breeders.
  - Early field results appear to validate rust-screening performance for percent stem symptoms.
    - Data from future assessments will enable correlation between resistance traits observed in a single inoculation of 2-year old material (e.g. needle lesion frequency, bark reaction, prevention of stem infection) and field performance (e.g. low infection frequency, inactivation of stem infections, and tolerance)
  - Although SP and WWP both have major genes for resistance (Cr1 and Cr2, respectively), virulent strains of rust are known that overcome this resistance on a few sites. For restoration and reforestation, this major gene resistance will be coupled with other types of resistance.
- **Orchards and Breeding**
  - Orchards have been established from resistant selections. Many of the first selections are at or near reproductive age, and breeding is underway.
  - Resistant seed is available for many breeding zones.
  - Breeding resistant selections will increase the utility and durability of partial resistance traits and raise the current level of resistance beyond that in natural populations.
- **Future Efforts**
  - A project to summarize the major findings from previous screenings and to help refine future screening and breeding efforts is in progress.
  - Research efforts are needed in areas such as physiology of resistance, confirmation of underlying mechanisms and their inheritance, and resistance x environment interactions, but reduced staffing and budgets may slow these efforts.
  - The Region 6 program has recently begun testing of whitebark pine for major gene resistance as well as for other resistance traits. Due to the urgency of the threat to whitebark pine, screening efforts are likely to increase.

Bark reaction on stem of sugar pine at a field validation trial

Western white pine seedling with resistant (hypersensitive response) and susceptible spots in major gene resistance screening trial

Acknowledgments: The Forest Service, Bureau of Land Management, and other cooperators have made the many phenotypic selections and supplied seed for disease testing. Region 1 supplied seed from 5 full-sib checklots. The Region 6 Genetics Program and Forest Health Program have provided funding. Researchers in Idaho and California have provided many insights into the genetics of blister rust resistance.

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